

UAMS I03-04

EV 316331604 US

What is claimed is:

1. An isolated nucleic acid for detection of *H. capsulatum* comprising:
- (a) SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6; or
- (b) the complement of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6.
- (c) a fragment of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or a fragment of the complement of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 that hybridizes under highly stringent conditions to at least one *H. capsulatum* chitin synthase intron sequence.
2. The isolated nucleic acid of claim 1, wherein said fragment comprises at least 8 consecutive nucleotides of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6, or the complement of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6.
3. The isolated nucleic acid of claim 1, further comprising an oligonucleotide having the nucleic acid sequence SEQ ID NO: 7 or SEQ ID NO: 8.
4. An isolated nucleic acid for detection of *H. capsulatum* comprising:
- (a) the nucleotide sequences set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or any complements thereof;
- (b) a nucleotide sequence having at least 70% sequence identity to any one of the sequences in (a); and
- (c) a fragment of any one of (a) or (b).
5. A method for detecting *H. capsulatum* in a sample, comprising the steps of:
- (a) providing a sample; and

UAMS I03-04

EV 316331604 US

(b) assaying for the presence of DNA comprising a *H. capsulatum* chitin synthase gene in said sample, wherein the presence of said chitin synthase DNA indicates that the sample contains *H. capsulatum*.

6. The method of claim 5, wherein the intron 1 of the *H. capsulatum* chitin synthase 2 gene is assayed.

7. The method of claim 5, wherein the sample is obtained from a human.

8. The method of claim 5, further comprising the steps of:

(a) exposing the sample under high stringency hybridization conditions to at least one isolated nucleic acid that hybridizes to at least one intron of the *H. capsulatum* chitin synthase 2 gene; and

(b) determining whether there is hybridization of the isolated nucleic acid to the sample, wherein a sample comprising *H. capsulatum* exhibits detectable hybridization and a sample lacking *H. capsulatum* does not exhibit hybridization.

9. The method of claim 8, wherein the isolated nucleic acid comprises:

(a) the nucleotide sequences set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6, or any complement thereof;

(b) a nucleotide sequence having at least 70% sequence identity to any one of the sequences in (a); and

(c) a fragment of any one of (a) or (b).

10. The method of claim 5, further comprising the steps of:

(a) conducting polymerase chain reaction (PCR) amplification using at least one nucleic acid primer that hybridizes to at least one intron of the *H. capsulatum* chitin synthase 2 gene; and

(b) determining the presence or absence of the PCR product resulting from said amplification.

UAMS I03-04

EV 316331604 US

11. The method of claim 10, wherein the primers hybridize to intron 1 of the *H. capsulatum* chitin synthase 2 gene.

12. The method of claim 10, wherein the primers comprise at least one oligonucleotide having the sequence SEQ ID NO: 7 or SEQ ID NO: 8.

13. A method for detecting an active case of histoplasmosis in a sample, comprising the steps of

(a) providing a sample; and

(b) assaying the sample for the presence of *H. capsulatum* chitin synthase mRNA or any fragment thereof wherein detection of *H. capsulatum* chitin synthase mRNA is associated with an active case of histoplasmosis.

14. The method of claim 13, further including the steps of:

(a) exposing the sample under high stringency conditions to at least one isolated nucleic acid that hybridizes to *H. capsulatum* chitin synthase mRNA or any fragment thereof; and

(b) determining the levels of *H. capsulatum* chitin synthase mRNA based on the amount of hybridization.

15. The method of claim 13, further including the steps of

(a) preparing *H. capsulatum* chitin synthase cDNA using mRNA from the sample as a template;

(b) conducting PCR using primers that hybridize to the *H. capsulatum* chitin synthase 2 cDNA; and

(c) ascertaining the presence or absence of product, wherein detection of the amplification product is associated an active case of histoplasmosis.

16. The method of claim 15, wherein the primers comprise at least one oligonucleotide having the sequence SEQ ID NO: 15 or SEQ ID NO: 16.

UAMS I03-04

EV 316331604 US

17. A kit for detection of *H. capsulatum* comprising:

(a) one or more containers comprising at least one oligonucleotide primer or DNA probe comprising sequences that hybridize to at least one intron of a *H. capsulatum* chitin synthase gene; and

(b) at least one separate container comprising *H. capsulatum* DNA comprising chitin synthase intron DNA complementary to said primers.

18. The kit of claim 17, wherein the intron is intron 1 of the chitin synthase 2 gene.

19. A method for using molecular genetic techniques to provide a strain of *H. capsulatum* comprising reduced pathogenicity by preparing *H. capsulatum* in which chitin synthase gene expression is either repressed or altered such that production of functional chitin synthase protein is significantly reduced.

20. The method of claim 19, wherein the chitin synthase gene is placed under control of a repressible promoter.

21. The method of claim 19 wherein chitin synthase gene expression is permanently repressed.

22. The method of claim 19, comprising production of *H. capsulatum* strains comprising a disrupted chitin synthase genomic sequence.

23. The method of claim 18, wherein the strain comprising reduced pathogenicity is used to provide a vaccine against *H. capsulatum*.

24. *H. capsulatum* strains made by the method of claim 18.

25. A method for inhibiting *H. capsulatum* chitin synthase production comprising generating a small inhibitory RNA that binds to and prevents expression of the *H. capsulatum* chitin synthase 2 gene and adding said RNA to a cell.

UAMS I03-04

EV 316331604 US

26. A composition comprising a small inhibitory RNA made by the method of claim 25.

5